Research Priorities in Large Bowel Cancer Prevention

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CANCER of the large bowel is one of the most common malignancies in industrialized countries. In 1990, this cancer will kill an estimated 60,900 people in the United States, making it the second leading cause of cancer death.[1] An estimated 155,000 men and women in this country will be diagnosed with large bowel cancer this year, with only 50% surviving more than 5 years.[1]

Several screening strategies to reduce mortality from this disease, including detection of occult fecal blood[2] and flexible sigmoidoscopy, have been proposed.[3] However, the value of available screening modalities in reducing cancer mortality is still the subject of intensive debate and research.[4]

Given that available treatment is of limited value in many patients with unresectable large bowel cancer and that screening has an uncertain role in controlling this disease, the need for primary prevention initiatives is great. Even though the causes of large bowel cancer are understood far less clearly than the causes of lung cancer, research over the past few decades points to realistic possibilities for reducing the incidence of large bowel malignancies.

THE CONTINUUM OF LARGE BOWEL CARCINOGENESIS

Figure 1 suggests a dynamic continuum of events leading to large bowel cancer. The process begins with one or more environmental factors acting in concert with one or more host factors. This complex of environmental and host factors leads to changes in the internal large bowel environment, which in turn effect specific alterations in the large bowel that are necessary precursors to the development of a cancer.

Large bowel carcinogenesis originates in a long-term, evolving interaction between environmental and host factors. For example, dietary factors may alter endogenous characteristics such as bowel flora or hepatic metabolism, which in turn may influence the further processing of ingested foods and carcinogens. However, the extent to which dietary factors alter gut flora or liver functioning may in turn be determined by inherited metabolic characteristics or prior exposure to environmental factors such as ethanol or occupational toxins. Given the complex processes leading to large bowel cancer, the responsibility of investigators in cancer prevention is to identify those elements of the environment-host interaction that are decisive, that is, are most amenable to practical interventions leading to a reduction in the incidence of this disease.

Although the relation between environmental and host factors is dynamic and mutually reinforcing, this discussion will deal with these two sets of factors separately.

ENVIRONMENTAL FACTORS

Evidence for Environmental Determinants of Large Bowel Cancer

There is more than a tenfold difference in colon cancer mortality between those countries with the highest and those with the lowest rates. Results from time-trend and migration studies indicate that these geographic differences are attributable primarily to environmental factors. From 1969 to 1981 the large bowel cancer mortality in Japan increased 44% in men and 40% in women.[5] Numerous studies of cancer rates in migrants demonstrate that the large bowel cancer rates of migrants generally converge on the rates for the country of destination, even for countries in which rates were initially higher.[6] It is particularly noteworthy that this convergence of rates can occur within the lifespan of the migrants themselves.[7]

These marked changes in disease rates over a relatively short period likely reflect changes in the exposure environment rather than any alteration in inherited susceptibility to large bowel cancer.

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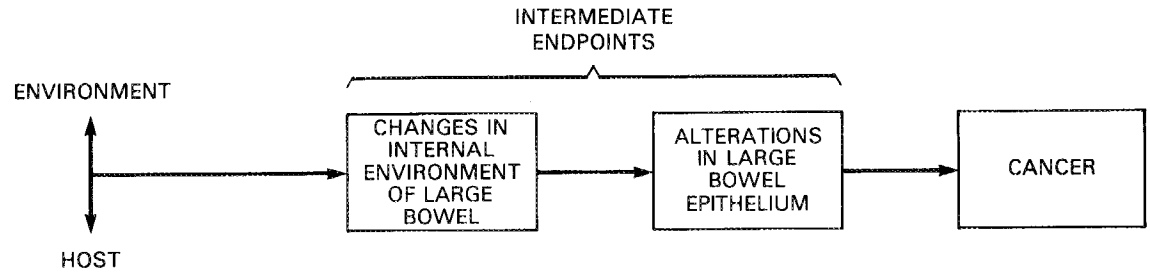

Fig 1. Continuum of large bowel carcinogenesis.

Evidence in Support of Dietary Causation

On both epidemiologic and physiological grounds, it is reasonable to suspect that dietary factors play a key role in the etiology of large bowel cancer. In accord with the ecologic evidence, dietary patterns vary widely across countries; dietary intake has changed over time in several countries such as Japan; and diet certainly changes with migration and acculturation.[8,9] Diet as an etiologic factor is certainly plausible biologically. Food and its various metabolites reach the bowel mucosa directly, and various nutrients affect several physiological processes that may be important in large bowel carcinogenesis, including bile acid production and pH determination.[10,11]

Researchers have identified relatively few nondietary, environmental determinants of large bowel cancer. Most studies have not found smoking to be related, and the data implicating occupational factors (such as asbestos) are sparse.[12]

Specific Dietary Hypotheses

The following sections briefly review the leading dietary hypotheses for large bowel cancer.

Dietary fat. Countries with higher per capita fat consumption tend to have higher large bowel cancer rates, with correlation coefficients in the range of 0.8.[8] Several analytic epidemiologic studies have shown a positive association between fat intake and large bowel cancer, though the data are not entirely consistent.[13] In a recent prospective study comprising 150 colon cancer cases among nurses, Willett[14] found a relative risk of 1.9 for women consuming 65 g or more of animal fat daily (highest quintile) compared with those consuming less than 39 g (lowest quintile). No association was noted between vegetable fat and colon cancer in these women. Experiments involving the administration of chemical carcinogens to laboratory animals have also shown dietary fat to be a potent promoting agent.[15]

One means by which dietary fat may affect large bowel carcinogenesis is through its influence on bile acid production.[16,17] In laboratory models, bile acids, especially the secondary bile acids, alter the proliferative activity of intestinal crypt cells[18] and promote tumorigenesis.[19] In addition, the amount of fat in the diet directly affects the amount of free fatty acids in the bowel lumen,[20] and these free fatty acids may also damage bowel mucosa.[21]

Calories. It is difficult to separate the effects of high-fat and high-caloric intake. Foods high in fat also tend to be high in calories, because fats (9 kcal/g) are more calorie-dense than proteins or carbohydrates (4 kcal/g). Increased caloric intake increases colorectal tumor yield in animal experiments,[22] and several case-control investigations have noted a crude association of total calories with risk of large bowel cancer.[23] However, Willett et al observed no effect of total energy consumption in their recent prospective study of colon cancer,[14] and, in at least one large case-control study, the crude effect of caloric intake disappeared when fat consumption was taken into account.[24]

Meat. Another correlate of fat intake that may influence the risk of large bowel cancer is meat consumption. Cooked meats are a major source of animal fat and calories, but they also contain known carcinogenic compounds, including a class of heterocyclic amines that are produced with high-temperature cooking methods such as broiling and frying.[25] Although it is known that human populations consuming large per capita quantities of meat are also routinely ingesting an appreciable quantity of mammalian

carcinogens derived from cooked meats, the effects on humans are still unknown. The two epidemiologic studies that examined cooked meat consumption and large bowel cancer risk did not observe an association,[14,26] but the strength of laboratory evidence warrants further epidemiologic investigations on this topic.

Dietary fiber. Burkitt[27] suggested 16 years ago that a relative deficit of fiber in the diet typical of Western industrialized countries was responsible for elevated large bowel cancer rates in these countries. The international correlation data support the dietary fiber hypothesis, with countries having a greater per capita consumption of dietary fiber having lower large bowel cancer mortality,[28] although the correlations are not as strong as those for dietary fat.[8] Findings from within-country interregional studies also corroborate this hypothesis.[29] A majority of analytic epidemiologic studies that assess dietary fiber have generally shown a protective effect for fiber.[30] Experiments in animal models have demonstrated a protective effect of insoluble dietary fiber, but results have been inconsistent for other types of fiber.[31]

The dietary fiber hypothesis, like the dietary fat hypothesis, is also biologically plausible. Dietary fiber can be defined on a physiological basis as the endogenous components of plant materials in the diet that are resistant to digestion by enzymes produced by humans.[32] The major dietary components are the nonstarch polysaccharides, cellulose, hemicellulose, and pectin, plus the nonpolysaccharide, lignin. By increasing stool bulk, dietary fiber is thought to dilute the concentration of potentially carcinogenic or mucosa-damaging substances (such as bile acids) in the bowel lumen.[33] Moreover, ingestion of some types of fiber increases fermentation by gut bacteria. This, in turn, produces short-chain fatty acids in the bowel and reduces the intraluminal pH.[34] A lower pH may reduce bowel cancer risk by reducing the solubility and ionization of both free fatty acids and free bile acids—the ionized form of these substances results in mucosal damage.[10] In addition, the short-chain fatty acid butyrate may have antineoplastic properties of its own.[35]

Vegetables and fruits. Case-control studies of large bowel cancer have assessed vegetable intake more frequently than any other nutrient or food group; it is usually found to be protective.[36] Although earlier case-control studies reported the protection to be due to specific cruciferous vegetables, more recent studies have reported a reduced risk for all vegetables.[36] Because vegetables in most industrialized countries are a major source of dietary fiber (42% in the United States[37]), it is often difficult to separate the effect of fiber and vegetables in analytical studies. Thus, the protection from vegetables might also be due to fiber or the combination of fiber and specific anticarcinogens.

Although the association between fruit intake and large bowel cancer is not as strong as that for vegetable intake, researchers have reported a protective effect in several case-control studies.[38-40]

Animal experiments have been largely limited to isolated chemical constituents of vegetables, rather than vegetables per se.[41] Vegetables contain cancer inhibitors such as indoles, flavonoids, and glucosinolates.[42] Several potential anticarcinogenic constituents in vegetables are also present in fruits.[43]

Calcium. Garland et al,[44] in the prospective Western Electric Study, have shown an inverse relation between dietary calcium intake and subsequent colorectal cancer. Epidemiologists have found this calcium-large bowel cancer link in other, but not all, studies.[45] Although the number of data points was small, an international correlation study has shown an inverse relation between dietary calcium intake and large bowel cancer incidence.[45] Calcium supplementation in human volunteers can reduce the proliferative activity of colonic epithelial cells[46,47] although a recent study did not replicate this finding.[48]

Calcium may bind intraluminally with free fatty acids and free bile acids to prevent mucosal damage.[20] In support of this idea, Wargovich et al[49] have shown that calcium prevents mucosal damage from experimentally instilled fatty[49] and bile[50] acids in animal models. However, not all the relevant effects of calcium need be intraluminal, and a systemic effect (with calcium reaching colonic epithelial cells through the blood) is plausible.[34]

Alcohol. Although several studies have demonstrated an association between alcohol consumption and large bowel cancer, particularly between beer and rectal cancer, other studies

have shown no such relation.[51] The inconsistency in these studies to date does not permit any firm conclusions about a role for ethanol in the etiology of large bowel cancer.

Methodological Problems in Studying Diet and Large Bowel Cancer

Although many researchers accept the importance of diet as the key environmental determinant of large bowel cancer, progress in the development of specific primary preventive strategies is constrained by a few persistent methodological problems.

The concept of dietary pattern. People do not eat single nutrients in isolation; they eat a mix of whole foods. Moreover, the various nutrients associated with large bowel cancer are highly correlated in human diets. As a consequence of this intercorrelation of nutrients, it is difficult to disentangle the effects of animal fat versus total calories versus cooked meat or of fiber versus vegetables and fruit in analytic epidemiologic studies. This intercorrelation of key nutrients argues for a greater emphasis—in both observational and intervention studies—on the concept of "dietary pattern." A dietary pattern can be characterized by a particular cluster of several key nutrients, but it comprises the totality of foods. Examples of dietary pattern might be the "typical Western high-fat diet" or the "Middle Eastern diet." It is possible to identify dietary patterns in this country that correspond—at least in terms of several key dietary elements—to those from low- and high-risk areas around the world.

Apart from its practicality, the dietary pattern concept has other theoretical virtues. There may be unidentified nutrient components of a dietary pattern that play an important etiologic role in large bowel cancer. Etiologic analyses or interventions based on dietary patterns will necessarily capture the biological effects of these unidentified nutrients that might be missed in investigations focusing only on specific (and known) nutrients. Furthermore, there may be important and potentially complex interactions among dietary constituents in relation to large bowel carcinogenesis. These interactions will be automatically reflected in any association demonstrated between a dietary pattern and large bowel cancer, but they may be difficult to demonstrate when the research focus is a single nutrient.

A few epidemiologic studies have assessed the effect of a combination of dietary variables on colon cancer risk; for example, a high-fat/low-fiber diet has been associated with an increased risk.[52] Kune et al have recently found a 20-fold difference in large bowel cancer risk for persons with six or more dietary risk factors compared to those with one dietary risk factor.[39]

Limitations of animal models. Although most animal carcinogenicity experiments have been conducted in rodents, there is some concern that rodents do not provide an optimal animal model for human large bowel carcinogenesis. Unlike humans, rodents have large cecums with intense bacterial metabolic action. Their bacterial flora are quite different from human colonic microflora and produce different metabolic products.[53]

Consequently, some investigators are conducting experiments in primates, particularly cotton-topped tamarins, that are prone to adenoma formation, colitis, and colorectal cancer.[54] For a few key questions, these experiments may yield more directly applicable results than rodent studies.

Attempts also have been made to develop more specific rodent bioassays that might prove predictive for the detection of large bowel carcinogens.[10] These are not yet widely used or adequately validated.

Error in dietary assessment. Several methods are used to assess what people eat. The usual goal of an epidemiologic investigation of diet and a disease outcome is to determine "typical" or "average" intake of various foods and nutrients and then to classify individuals into one of several categories of intake. Investigators then determine the disease risk associated with each of these intake categories.

The most common techniques for assessing diet in epidemiological studies are the 24-hour recall, the food frequency questionnaire, and dietary records kept over several days.[55] No matter which approach is used, food intake measurements contain considerable error. A person may not remember what he or she ate or how the food was prepared, a problem exacerbated when the respondent did not prepare the meal. Even with models and photographs to help study

participants, the estimation of portion size may be inexact. The considerable variability in day-to-day intake of particular foods and nutrients means that substantial error may accompany a 24-hour recall estimate of food intake.

The consequence of this measurement error in dietary assessment is that some people are misclassified, that is, placed in an incorrect (with respect to what was truly eaten) category of intake. As a result, the observed relative risk for a given category of intake is attenuated compared with the "true" relative risk.[56,57]

Moreover, it is entirely plausible that nutritional factors may be important during earlier life. Because diet assessed in later life is, at best, only partially correlated with earlier life diet, the true relative risks for earlier life diet are likely to be markedly attenuated when estimated on the basis of diet assessed in later life. Investigators are attempting to develop and validate questionnaires that would permit assessment of early diet, but an accurate assessment of earlier life diet may remain elusive for some time.

There has been a great deal of interest in developing "hard" biological markers of dietary exposure, but work in this area is still preliminary. Potential markers of fat intake include serum cholesterol, aggregate serum fatty acid profiles, and cheek or adipose cell fatty acids, but there is no definitive marker of fat intake. Serum carotenoids and fecal fiber may be of value in assessing the intake of fruits and vegetables and other high-fiber foods. It may be possible to develop markers that reflect an overall dietary pattern more accurately than single nutrients. For example, serum cholesterol may correlate more strongly with a low-fat/high-fiber diet than with a low-fat diet alone. The use of biological dietary exposure markers in case-control studies is problematic, because both the disease and its treatment may affect the value of these markers.

Dietary homogeneity. Another potential methodological difficulty in epidemiologic studies of diet and large bowel cancer is the relative homogeneity of diet within the countries or regions where studies are carried out. The 10-fold cross-national range in large bowel cancer rates corresponds to a range in, for example, dietary fat intake of 18% to 42% calories from fat and 10 to 50 g of total dietary fiber a day. The range of nutrients such as fat and fiber within the US population is considerably less than the international range; only about 5% of the US population reports an intake of less than 25% calories from fat,[58] and only 10% of the population reports a consumption of more than 20 g of fiber daily.[59] Therefore, the relative risk for those in the highest versus the lowest categories of intake for fat and fiber will be considerably lower than 5 (corresponding to the international variation in large bowel cancer rates). Epidemiologic studies have to be quite large to have sufficient statistical power to detect relative risks in the range of, say, 1.5 to 2.0, but this may well be the magnitude of risk that prevails within countries with relatively homogeneous diets. The homogeneity problem may be avoided to some degree by conducting studies in areas with populations having relatively diverse diets.

HOST FACTORS IN THE ETIOLOGY OF LARGE BOWEL CANCER

Host factors may confer differential susceptibility to large bowel cancer and may be either inherited or acquired.

A genetic predisposition to large bowel cancer has been well documented in patients with familial polyposis coli, a rare syndrome characterized by multiple colorectal polyps, which occurs with a population frequency of approximately 1 in 10,000.[12] By age 30 about 50% of these patients will have developed large bowel cancer, and by age 50 the incidence of colorectal cancer approaches 100%. Less striking predispositions to large bowel cancer are seen in other inherited polyposis syndromes and in patients with the "cancer family syndrome," who exhibit multiple adenocarcinomas, particularly of the colon and endometrium.[60] There is also evidence for a familial component to the risk of so-called "sporadic" large bowel cancers, with a family history of colon cancer in a first-degree relative conveying a threefold increase in risk.[61]

The explanation for familial aggregations of large bowel cancer may be genetic, as suggested in a recent analysis of Utah kindreds, which supported a dominant pattern of inheritance for susceptibility to adenomatous polyps and large bowel cancer.[61] It is possible that one genetically controlled mechanism affecting susceptibility may involve varying metabolic phenotypes in the activation and excretion of ingested carcinogens.[62]

However, familial mechanisms need not be genetic. Early shared diet could influence risk, perhaps via the establishment of specific fecal floral populations that are resistant to subsequent change.[53]

Another well-documented example of an acquired host factor might be the predisposing medical condition ulcerative colitis (the development of which may in turn represent genetic influences as well). The risk of large bowel cancer in ulcerative colitis patients is high, reaching 50% incidence after 30 years with the disease.

Both acquired and inherited factors can modify one another. Colonic surgery, for example, has been shown to reduce the incidence of rectal adenomas in familial polyposis.[63]

The reciprocity of the environment-host relation is reflected in potential risk factors such as lack of exercise,[64] low parity,[65] and obesity.[66] These factors have often been regarded as environmental "exposures," but they can also be considered as host characteristics.

INTERMEDIATE ENDPOINTS

Studies of the microlevel processes (mechanisms) potentially involved in the genesis of large bowel malignancies have been reviewed elsewhere.[9-11] Investigations of mechanisms can enrich understanding of large bowel carcinogenesis and direct attention to new preventive strategies (carried out at the population or clinical level).

Diet influences several bowel processes with possible etiologic significance. According to the model of a continuum of large bowel carcinogenesis (Fig 1), these internal processes (intermediate endpoints) can be divided into two categories: general changes in the internal large bowel environment and specific changes in the large bowel mucosa.

Changes in the Internal Environment of the Large Bowel

The following changes in the internal bowel environment are potential links in the causal pathway to cancer.

Fecal mutagenicity. In the attempt to link dietary carcinogens and risk of large bowel cancer, an intermediate endpoint of great intuitive appeal is fecal mutagenicity. Correlational studies have indicated that fecal mutagenicity is elevated in populations who consume high-fat, low-fiber diets and are known to be at high risk for large bowel cancer.[67] These observations have suggested the possibility of identifying fecal carcinogens that cause large bowel cancer while in passage through the bowel. However, optimism in this research area has been tempered by the discovery that not all fecal mutagens are dietary in origin or even carcinogenic.[68] Future work will likely focus on the effects of specific mutagenic fractions or compounds such as cooked meat metabolites.[69]

Bile acid-neutral sterol excretion. The role of bile acid and neutral sterol metabolism as modulators of colorectal cancer continues to be debated with inconclusive results. The laboratory evidence supporting a role for bile acids in large bowel carcinogenesis has been reviewed elsewhere.[11] As one example of a possible etiologic mechanism, the secondary bile acids (deoxycholic and lithocholic acids), produced by the action of bowel flora on the primary bile acids released from the liver into the intestine, have been shown to irritate the mucosa of the large bowel.[50] Because inflammation is followed by increased cell turnover as part of mucosal repair, there is some reason to suspect that excessive excretion of secondary bile acids may promote large bowel tumorigenesis.

However, no specific measure of secondary (or any) bile acid excretion consistently relates to risk of large bowel cancer. Similarly, investigators have postulated that the fecal concentrations of various cholesterol metabolites, the neutral sterols, affect risk but pertinent epidemiologic studies have been inconsistent.[10,70] Some of this confusion may reflect the confounding effects of colorectal bleeding, which is very common in patients with large bowel cancer and which dramatically affects fecal bile acid and neutral sterol measurements.[71] Thus, the relation of these compounds to risk may not be reliably observable with the case-control study designs that have been used. However, it has proven relatively uninformative to study patients with cholecystectomies who have increased enterohepatic circulation of bile. Investigators observing an increased risk of subsequent large bowel cancer cannot disentangle true risk from the possibility of detection bias related to increased postoperative surveillance.[72] A promising current line of inves-

tigation is the follow-up of large cohorts of patients taking serum cholesterol-lowering medications that may increase fecal bile acid and neutral sterol excretion.

Bowel flora. In research related to bile acid and neutral sterol metabolism, investigators have looked for patterns in colonic microfloral populations or bacterial metabolic activity that might signal an increased risk of large bowel cancer.[73] At least superficially, the epidemiology of bowel cancer supports an important role for the colonic microflora, with cancer rarely occurring in the relatively germ-free small intestine but commonly in the contiguous large bowel where plentiful microflora contribute much of the dry stool weight. However, no firm conclusions have been reached in this research area, perhaps because of the great difficulty of typifying the complex ecosystem of anaerobic organisms that populate the bowel.

pH. Some researchers have proposed that an elevated pH within the large bowel lumen is a risk factor for large bowel cancer.[10] Several ecologic studies have shown that fecal pH is higher in those geographic areas or among population groups with higher colon cancer incidence relative to those with lower colon cancer incidence.[74] Two small case-control studies have shown a higher pH in the feces of cases compared with that of controls.[75,76]

pH may influence large bowel carcinogenesis by its effect on lipids within the colonic lumen[34]; a small percentage of dietary fat is not absorbed and appears in the feces as free fatty acids. Similarly, a small amount of bile avoids the normal reabsorption associated with the enterohepatic circulation in the small intestine and appears as free bile acids in the large bowel lumen. Ionized free bile and free fatty acids are irritating and toxic to large bowel epithelial cells, and the proportion of the free bile and fatty acids in the ionized state rises as the intraluminal pH increases.[34] In support of these theoretical considerations, one animal study has shown that the damaging effect of ionized lipids is pH-dependent, with little effect at a pH of 5.9 and much more extensive damage at a pH of 7.9.[77]

Specific Alterations in Large Bowel Epithelium

Intermediate endpoints that involve a specific alteration—at the organ, tissue, cell, or molecular level—may affect the structure and/or function of the large bowel.

DNA adducts. An intermediate endpoint that has stimulated great general interest is the formation of DNA adducts, the binding of putative carcinogens to cellular DNA.[78] Conceptually, DNA adducts can be viewed as the ultimate measurement of carcinogenic exposure, of the biologically effective dose. But this concept may be naive because exposures to accepted carcinogens (such as benzopyrene from cigarette smoke) have resulted in high levels of DNA adducts not only in "target" tissues (such as the lung) but also in tissues resistant to tumor formation (such as heart muscle).[79] DNA adducts are difficult to measure. Current assays depend on the collection of tissue samples and require a great deal of time and technical expertise. Nonetheless, there is enthusiasm for exploring the possible significance of DNA adducts as intermediate endpoints. With regard to large bowel carcinogenesis, a feeding experiment in primates recently showed DNA adducts in the colon mucosa (among other tissues) following administration of one of the heterocyclic amines found in cooked meats, a compound that had already been shown to produce tumors in rodents.[78] The relevance of this result will be more clear once ongoing carcinogenicity experiments in the same species of primate are completed.

Chromosome alterations and oncogenes. Recently, an expanding set of specific chromosomal abnormalities has been documented in patients with increasingly severe colorectal adenomatous polyps and cancer.[80] These alterations include allelic deletions and *ras* -gene mutations, and the presence of multiple concurrent abnormalities has been associated with a high probability of severe disease and a worse prognosis. It is unclear whether these abnormalities represent the cause or effect of worsening neoplasia. At a minimum, an appreciation of these common chromosomal alterations may prove useful for more accurately placing patients with polyps on the natural history continuum from benign to malignant disease.

Researchers have detected altered expression of several oncogenes as well as endogenous retroviral-like DNA sequences in large bowel neoplasms.[81] Although it is plausible that the structural modification or inappropriate expres-

sion of oncogenes has a causal role in large bowel carcinogenesis, these molecular changes could also represent merely the consequences of various stages of malignant transformation.

Cell kinetics/proliferation/muscosal damage. One of the most promising intermediate endpoints involves proliferation of large bowel epithelial cells.[82] The normal proliferative region of the large intestine is found in the lower two thirds of the colorectal crypts. Cells migrate up the crypts to cover the crypt surface and are exfoliated from the mucosal surface in 3 to 8 days in humans. As the cells migrate upward, they mature and lose their ability to proliferate. Autoradiographic techniques using tritiated thymidine have been used to characterize epithelial cell kinetics. The labeling index referred to in studies using this technique represents the proportion of cells within a crypt, or at a given height along the crypt, that are undergoing DNA synthesis (ie, are in the S-phase of the cell cycle).[83] Thus, although this assay is not a direct measure of the formation of new cells,[84] it is likely to be a proxy for cell proliferation.

The precise role of hyperproliferation in large bowel carcinogenesis remains to be determined. It is possible that specific environmental exposures (including but not necessarily restricted to genotoxins) lead to hyperproliferation, which leads to neoplasm formation. Alternatively, certain genotoxins may act only in the presence of a hyperproliferative state induced by environmental (particularly dietary) or genetic factors.

Several studies have now shown that the labeling index is higher in persons from populations with a higher incidence of large bowel cancer compared to persons from populations with lower incidence rates.[82] The labeling index has also been shown to be higher in the normal mucosa of subjects with a history of cancer or adenomas. In addition to the human studies of the effect of calcium carbonate supplementation on the labeling index,[46-48] a recent study has shown that a bolus of corn oil increases the index.[84]

Other mucosal changes that could serve as intermediate endpoints. Another possibly useful measure of cell proliferation is the tissue level of ornithine decarboxylase, a rate-limiting enzyme in the biosynthesis of polyamines associated with cell growth.[85] Increased ornithine decarboxylase activity has been observed in the large bowel mucosa of patients with colonic polyps or cancer,[86] and high levels of ornithine decarboxylase may indicate mucosal proliferation and increased cancer risk. No large epidemiologic studies of this possible intermediate endpoint have been reported, probably because current assays require colonic cell samples obtainable at present only by mucosal tissue biopsies, ruling out the general testing of asymptomatic controls.

Another suggested marker of early large bowel neoplasia is an alteration in the biochemistry of the mucins normally produced by colonic mucosal cells. Certain mucin-associated antigens, rarely expressed by normal colonocytes, are frequently expressed by malignant, premalignant, and to some extent hyperplastic colonic mucosa.[87] One group has proposed a colorimetric assay that may permit the detection of altered colonic mucins.[88] If validated in prospective studies, this simple test of a mucus sample obtained on routine rectal examination could be used in population studies.

Validation of Intermediate Endpoints

Figure 1 shows the direct causal linkage from environment-host through intermediate endpoints to cancer. The alteration in the intermediate endpoint is a necessary step in the development of cancer, that is, exposure works through the intermediate endpoint. The goal of intermediate endpoint research has been to discover an intermediate endpoint that could serve as a proxy for neoplasia so that an experimental manipulation of the endpoint would mean that the same manipulation would have a similar impact on neoplasia. Only if the intermediate endpoint is in—or is tightly linked to—the causal pathway to cancer, as shown in Fig 1, would the intermediate endpoint serve this purpose.

However, a given biomarker may be associated with large bowel cancer but not be a necessary step in the causal pathway to cancer. In particular, two noncausal relations between a marker and cancer need to be considered.

First, an environmental exposure may affect both the marker and cancer, but with the marker having no causal relation to cancer. The marker will be perceived as being correlated with cancer (eg, populations at high risk of cancer will tend to have higher values of the marker). An intervention that modifies the environmental exposure

will change the marker but need not have any influence whatsoever on the development of cancer.

Second, changes in a given marker may be the consequence rather than the cause of large bowel cancer. In that case, specific interventions designed to "counteract" what are really disease-induced changes in the marker need not have any impact on the cancer.

A given intermediate endpoint is valuable only in so far as it relates to exposure(s) and neoplasia in the causal manner outlined previously. Validation studies designed to demonstrate these relations for a given intermediate endpoint are critical.

Adenomatous Polyps

Adenomatous polyps of the large bowel present a unique intermediate endpoint in that they have a well-established link with cancer. (Although hyperplastic polyps are not considered adenomatous lesions, the word "polyp" is used here to refer to adenomas.)

The prevalence of one or more adenomas in middle-age and older adults is more than 30%, with a male predominance.[89] Autopsy studies indicate that the prevalence increases with age and may be as high as 50% or more in men and women older than 60.[90]

The epidemiology of polyps is extremely limited at present. Two small case-control studies of polyps (most of which were less than 2 cm in size) suggested a slightly lower intake of fiber and higher intake of saturated fat among cases.[91,92]

It is generally accepted that large bowel adenomas are a requisite precursor lesion for most large bowel cancers. Several lines of evidence support this idea of a polyp-cancer sequence,[90,93,94] including (1) cancer foci have been seen in polyps but not in normal mucosa; (2) residual polyp tissue has been found in small cancers; (3) some benign polyps have been shown to develop into cancers; (4) the proportion of polyps with cancer increases with increasing polyp size; (5) there is a similar anatomic distribution of polyps and cancer in the large bowel; (6) the geographic variation in polyp prevalence corresponds to that for cancer; and (7) the peak age of diagnosis for polyps precedes that for cancer by 5 years. Gilbertsen has shown that patients kept polyp-free remain cancer free, at least for rectosigmoid polyps.[95] A recent study of chromosomal aberrations demonstrates increasing chromosomal anomalies with increasing size and morphological atypia of colonic adenomas.[80] Moreover, all adenomas (tubular, tubulovillous, and villous) have been shown to have malignant potential.[93]

A particularly attractive feature of adenomas, from a research perspective, is that they have a high recurrence rate. ("Recurrence" here refers to the development of one or more polyps anywhere in the large bowel after prior removal of one or more polyps.) In retrospective endoscopy studies, annual adenoma recurrence rates of between 15% and 35% have been reported,[89,96-100] although some 10% of these "new" adenomas likely reflect missed polyps at the index endoscopy.[97] In a small randomized intervention study of the effect of vitamin C and E supplementation on large bowel polyp recurrence, McKeown-Eyssen et al observed an annual recurrence rate of 30%.[101] The high rate of polyp recurrence, at least 10% per year, means that an adequately powered intervention study could be carried out that is substantially smaller and shorter in duration (not to speak of less expensive) than an intervention trial with large bowel cancer as the endpoint.

Given the strong evidence for the polyp-cancer sequence, an intervention that reduces the recurrence of large bowel polyps would be highly likely to reduce the incidence of large bowel cancer.

FUTURE RESEARCH

The research logic developed here suggests several directions for future studies.

1. Animal experiments with manipulation of diets reflecting human dietary patterns (rather than single nutrients).
2. Continued efforts to develop "hard" biological markers of dietary intake, particularly for lipid, fiber, and vegetable and fruit intake. These efforts will aid in assessing what people truly eat.
3. Further observational epidemiological investigations of large bowel adenomatous polyps.
4. Intermediate endpoint validation studies.
   a. Animal experiments. To the extent that it is possible to perform intermediate endpoint assays without killing animals, then "complete" animal models can be

created in which diet is manipulated, the endpoint is assayed, and the animals are followed to tumor development.

b. Case-control studies, in which intermediate endpoint determinations are made for cases and controls. The case-control study design remains less expensive and faster than a prospective approach if issues of bias can be satisfactorily addressed. However, biased biological measurements in patients with large bowel cancer are difficult to avoid. Major concerns include tumor effects such as the influences of bleeding, effects of diagnostic procedures, treatment effects following bowel resection, and dietary modifications due to gastrointestinal disease with secondary changes in biological measurements. For all intermediate endpoints of interest, the investigator must address the possibility of disease affecting the measurements before suggesting a causal interpretation of any observed case-control differences. Despite methodological care, it is conceivable that some intermediate markers can only be validated using a prospective approach.

c. Cohort and intervention studies. The ideal approach to validating intermediate endpoints is to integrate them in prospective studies, either observational cohort studies or prevention trials, in which specimens are collected before the development of cancer (or polyps). Because only a relatively small proportion of study subjects will have a neoplastic endpoint, analyzing the data on all cases and only a fraction of the noncases (the nested case-control or case-cohort[102] design) is a reasonable way to proceed.

5. Analytic epidemiologic studies of large bowel cancer. In light of the inconsistency in previous epidemiologic studies of large bowel cancer, as well as some of the methodologic problems cited above, three suggestions may be of value.

First, if investigators are to make use of the efficiencies of the case-control design, it is essential to establish that retrospective dietary histories do not bias results. One approach to this problem is, in the context of ongoing cohort studies, to assess the diet of large bowel cancer cases and controls. These assessments can be compared with dietary data obtained at the cohort baseline.

Second, validation studies of the accuracy of dietary assessment should be built into the overall study.[103] If, for example, a food frequency questionnaire is the primary assessment instrument, dietary records can be collected over several days from a statistically appropriate sample of the overall cohort.

Third, studies should be large enough to compensate for both the error in dietary assessment and the dietary homogeneity problems. Countries with a fair amount of dietary diversity would be particularly good places for these studies, but even relatively dietarily homogeneous countries can be studied if the sample size is large enough to encompass a substantial number of persons at the "tails" of dietary intake for various nutrients. For cohort studies in which bias from retrospective dietary histories is not an issue, this may mean sample sizes of one hundred thousand or more. Such studies would undoubtedly be logistically complex and expensive—and probably few—but this is preferable to a large number of inconsistent and unconvincing smaller studies. It may well be possible to coordinate these cohort studies in advance, in effect establishing a kind of prospective meta analysis.

6. Adenomatous polyp recurrence trials. Intervention trials are major undertakings involving much effort. Why not just direct resources to additional laboratory investigations or observational epidemiology? The rationale for these trials is that they can address essential questions that cannot be answered by animal or analytic epidemiologic studies. These questions include (1) Will dietary change be effective in humans? (2) Will changes in an individual diet have an effect? (3) Will dietary changes be effective in the short-term? and (4) Will changes in adult life be effective? Furthermore, a major goal of research in the diet and large bowel cancer area is to provide a

scientific basis for recommending and implementing dietary changes. It is unlikely that any combination of further animal research, clinical investigations employing nonneoplastic endpoints, or observational epidemiological studies will be sufficiently persuasive to influence public health policy.[104] The Committee on Diet and Health of the National Academy of Sciences has recently concluded that "to obtain definitive information on the role of diet and cancer in humans, it would be desirable to conduct intervention trials in which diets are modified in specific ways. . . . Although intervention trials are likely to be very expensive, the magnitude of the health problem and the lack of satisfactory treatments for many major types of cancer warrant such an investment of human and financial resources."[104]

Several intervention studies involving supplementation with vitamins or calcium are under way. The completed study by McKeown-Eyssen et al shows a 17% reduction in polyp recurrence in the intervention group, but the study was not large enough to rule out this being a chance finding.[101] The National Cancer Institute is currently planning a multicenter intervention study to determine whether a low-fat, high-fiber, vegetable- and fruit-enriched dietary pattern will reduce polyp recurrence.

Although each type of investigation proposed here has the potential to advance understanding of large bowel cancer, the large cohort and polyp intervention studies are especially promising. In particular, should positive nutritional findings from these large cohort studies parallel those from the polyp trials, we will have come close to proving a causal link between diet and large bowel cancer and thereby providing a firm scientific foundation for prevention of this disease.

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